RESEARCH ARTICLE

Resistance reaction of soybean genotypes to *Spodoptera litura* under free-choice and no-choice tests and selection based on multiple traits

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ABSTRACT

Armyworm (*Spodoptera litura*) is the main leaf-damaging pest of soybean (*Glycine max* (L.) Merr.) plants. The research aims to assess the resistance response of soybean advanced lines against *S. litura*, evaluate their agronomic performance, and select the best genotypes based on multiple traits. The research was conducted in Malang, Indonesia. The evaluation for armyworm resistance was based on the free-choice test (FCT) and no-choice test (NCT). Each study utilized a randomized complete block design with 38 soybean genotypes and four replicates. The results showed that the NCT exerted a higher intensity of leaf damage and greater insect pest pressure compared to the FCT. Two soybean genotypes (G17 and G19) consistently demonstrated resistance to *S. litura* in both the FCT and NCT. Soybean seed yield has a significant positive correlation with plant height, number of filled pods, number of nodes, and number of branches, thus those characters could be used as indirect selection criteria for soybean yield. The selection based on GT Biplot revealed that G38 (resistant-control variety) and G17 had the best performance for resistance to *S. litura*, but did not exhibit significantly high seed yield. In contrast, G16 exhibited high yield performance but susceptibility to *S. litura*. The G36 was the best performer for the number of filled pods, number of nodes, number of branches, plant height, and 100-seed weight, despite being relatively less resistant to *S. litura*. G17 and G19 are promising candidates for breeding programs aimed at developing high-yielding and armyworm-resistant soybean varieties.

Key words: Agronomic character, correlation, genotype-by-trait biplot, seed yield, selection, soybean.

INTRODUCTION

Armyworm or common cutworm *Spodoptera litura* (Fabricius, 1775) is a leaf-feeding pest with a very wide distribution across different locations and crop commodities (EFSA Panel on Plant Health et al., 2019). In Indonesia, armyworm is a major leaf-feeding pest of soybeans (Fattah et al., 2018; Adie et al., 2020). Control strategies in soybean (*Glycine max* (L.) Merr.) production areas often rely on chemical or botanical insecticides. However, improper use of chemical insecticides can lead to environmental damage, harm to natural enemies, and the potential for resistance and resurgence in armyworm populations. An environmentally friendly alternative for controlling armyworm is through the use of resistant varieties in integrated pest management (IPM) systems (de Queiroz et al., 2020; Mathpal et al., 2022).

Researchers worldwide have investigated and evaluated soybean genotypes for their resistance to *S. litura*. Adie et al. (2020) assessed soybean genotypes and identified that two exhibited moderate resistance, while the remaining genotypes fell within the susceptible range. In another study, Mathpal et al. (2022) discovered four genotypes displaying high resistance, seven with moderate resistance, three susceptible genotypes, and two highly susceptible genotypes showing maximum feeding behavior. Furthermore, the evaluation of 150 soybean

genotypes identified Anjasmoro/Rajabasa-41 as resistant to *S. litura*, with specific traits such as low leaf water content and dense leaf trichomes contributing to its resistance (Krisnawati et al., 2021).

Recent research has also focused on evaluating resistance in various crops against armyworms. In tobacco, Samporis and Jimamwut showed moderate resistance to armyworm, while accessions DB Serongsong Sadhana and Paiton 1 were susceptible (Prabowo et al., 2021). Meanwhile, the fall armyworm (FAW) has emerged as a major threat to maize in recent years. A study reported that the damage severity caused by FAW ranged between 20% and 70% in monocultures and between 1% and 25% in the push-pull systems (Guera et al., 2023). Studies have demonstrated that the soybean genotype PI 227687 exhibits resistance to FAW, impacting larval development negatively when feeding on its leaves, especially older ones or those from reproductive-stage plants (de Souza et al., 2021). Furthermore, research by Sotelo-Cardona et al. (2021) highlighted FAW's preference for maize over other crops like cabbage and soybean for oviposition.

Based on the aforementioned research findings, it is evident that there are variations in the mechanisms of resistance to *S. litura* among different soybean genotypes. Oki et al. (2019) reported that wild soybean exhibits resistance to *S. litura*, attributing it to an antixenosis resistance quantitative trait locus (QTL) on chromosome 7, with the resistant allele derived from G406, whereas the Himeshirazu allele showed no antixenosis effect. In other study, the evaluation of soybean genotypes for *S. litura* resistance revealed that Tamahomare, Himeshirazu, and IAC100 showed significant antixenosis resistance, while Peking demonstrated a notably strong antixenosis effect for 3rd-5th instar larvae compared to 2nd instar larvae (Yano et al., 2021). In recent studies, Ongaratto et al. (2021) evaluated antixenosis resistance in soybean genotypes, focusing on parameters like oviposition behavior, attractiveness, and food consumption. Meanwhile, Bayu et al. (2018) identified nine genotypes showing antixenosis resistance to *S. litura*, indicating their potential as valuable genetic resources for enhancing soybean's defense against this pest. Furthermore, the soybean genotypes PI 227687, PI 227682, IAC 100, and BRS 7270 IPRO showed substantial antibiosis against *S. cosmioides* (de Queiroz et al., 2020), as evidenced by biological parameters. Du et al. (2022) utilized larval weight and the index of leaf damage as indicators of resistance against common cutworm. In other food crops, such as rice, it was indicated that the Miúdo Branco genotype exhibited antixenosis to *S. frugiperda*, whereas IR 64 and Bacaba Branco showed antibiosis effects by altering insect development and prolonging the insect life cycle (Silva et al., 2021). In maize, genotypes resistant to FAW are characterized by shorter development times and heavier larval and pupal weights (Navin et al., 2021).

The cultivation of insect-resistant soybean varieties has been effective in mitigating yield losses from armyworm attacks. This approach helps minimize plant damage, preserve natural enemy populations, and reduce reliance on insecticides for pest control, ultimately lowering production costs (Mathpal et al., 2022; Aryati and Ulina, 2023). Thus, the aims of the current study were to assess the resistance response of soybean advanced lines against *S. litura*, evaluate their agronomic performance, and select the best genotypes based on multiple traits.

MATERIALS AND METHODS

Plant genotypes and research location
The study used 37 soybean (*Glycine max* (L.) Merr.) advanced lines and one soybean genotype as resistant control. Experiments were carried out in Malang (08°11'02'' S, 112°33'32.9'' E; 445 m a.s.l.), East Java, Indonesia.

Armyworm rearing
The *Spodoptera litura* (Fabricius, 1775) were collected from soybean fields owned by local farmers in Malang, Indonesia. Collections were made in the form of larvae and eggs, which were then reared in plastic containers measuring 20 cm in diameter and 15 cm in height, covered with gauze. Each plastic container housed larvae and eggs separately. The diet provided consisted of castor (*Ricinus communis* L.) plant leaves, with feed replacement every 2 d. Once pupated, the pupae were transferred to plastic containers measuring 15 cm in length, 15 cm in width, and 6 cm in height, lined with tissue paper. Upon emergence from the pupae, the adults were transferred to gauze cages (15 cm in diameter, 30 cm in height) containing cotton soaked in a 10% honey solution as food for the adult moths, with bread paper lining provided for egg deposition. The eggs produced were further developed into larvae to establish the population used for the research.

Planting of soybean genotypes
The genotypes used in the studies were planted in 18 cm diameter plastic pots. Each pot was filled with a soil mixture enriched with organic fertilizer at a 1:1 ratio, with only one plant maintained per pot. The plants were fertilized using NPK fertilizer (15:15:15), applied 10 d after planting (DAP). Plant maintenance practices included optimal watering, weeding, and soil cultivation. Pest and disease control measures were initiated at 35 DAP and continued until maturity.

Research design and screening for *S. litura* resistance
The study consisted of two types of research: Free-choice test (FCT) and no-choice test (NCT). Each study utilized a randomized complete block design using 38 soybean genotypes with four replicates. Each plant was infested with two neonate *S. litura* larvae at 25 DAP. The FCT was conducted by placing 38 soybean genotypes (38 pots) inside a 3 m × 3 m × 3 m screen cage. Therefore, there were four screen cages in total, reflecting the number of replicates. The positions of the plants were randomly arranged, aiming for leaf contact between treatments so that *S. litura* larvae had the opportunity to choose leaves from preferred genotypes. In the NCT, each individual plant was individually covered with gauze. The wire cages used had 17 cm diameter and height of 80 cm. Randomization of the positions of the tested genotypes was performed for each replicate.

Data observation and statistical analysis
The observations were made on the agronomic traits (plant height, number of branches per plant, number of nodes per plant, number of filled pods per plant, number of empty pods per plant, 100 seed weight, and seed yield per plant) and leaf damage intensity. Leaf damage intensity was observed at 3, 6, 9, and 12 d after infestation (DAI).

The leaf damage intensity of *S. litura* was calculated using Equation 1 (Adie et al., 2020):

$$I = \sum \frac{n_i \times v_i}{Z \times N}$$

where I is leaf damage intensity, n_i is total number of leaves observed at v_i, v_i is score category (i = 0-4), N is total number of leaves, Z is the highest score (ranging from 0 to 4). The scoring criteria used were as follows: Score 0 = healthy leaves, score 1 = ≤ 25% of leaf area damaged, score 2 = > 25%-50% of leaf area damaged, score 3 = > 50%-75% of leaf area damaged.

The resistance level of soybean genotypes against armyworms was categorized based on the method developed by Chiang and Talekar (1980). The agronomic data were analyzed using ANOVA. Pearson correlation analysis was conducted to assess the magnitude of the correlation between the NCT and FCT, as well as among agronomic traits in both tests. These correlations were visualized in a correlogram generated with the corrplot function in the RStudio program (2020, RStudio, PBC, Boston, Massachusetts, USA). The which-won-where biplot from the Genotype by Trait (GT) biplot method (Yan and Rajcan, 2002) was used to visually represent the two-way data of treatment and traits in a biplot format. The GT biplot was applied for data from the NCT (seven agronomic traits and leaf damage intensity). All biplots presented in this study were generated using the "metan" R package within RStudio (2020).

RESULTS AND DISCUSSION

Leaf damage intensity in FCT and NCT
The leaf damage intensity of 38 soybean genotypes caused by *S. litura* in the NCT was higher compared to the FCT and remained consistent at 3, 6, 9, and 12 DAI (Figure 1). At 3 DAI, the damage intensity of *S. litura* in the NCT was 44.47%, whereas in the FCT it was 37.28%. Subsequently, it increased to 51.31% in the NCT and 44.16% in the FCT at 15 DAI. The average damage intensity of *S. litura* in the FCT at 3 and 6 DAI was similar and increased with the duration of *S. litura* infestation. Conversely, in the NCT, the damage intensity of *S. litura* at 12 and 15 DAI was comparable. The difference in leaf damage patterns caused by *S. litura* indicated that the increase in damage intensity in the FCT occurred from 12 to 15 DAI, while in the NCT it occurred from 3 to 9 DAI.
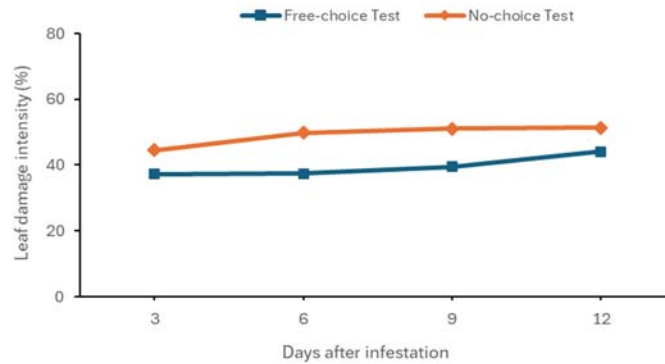
Figure 1. Leaf damage intensity of 38 soybean genotypes in the free-choice test and no-choice test.

The average leaf damage intensity of 38 soybean genotypes in the NCT was higher (49.17%), compared to the FCT (39.59%). Adie et al. (2020) also reported a similar finding where the intensity of leaf damage in the no-choice test was 49.07%, while in the choice test, it was only 28.94%. Additionally, a study with a similar method found that the NCT resulted in higher average intensities than the FCT (Krisnawati et al., 2022; Nuryati et al., 2023). This difference can be attributed to the fact that in the FCT, each insect has chance to select its host plants, whereas in the NCT, they are constrained to feed on the host plant without alternative options (Adie et al., 2020; Nuryati et al., 2023).

In this study, it was noted that the NCT method exerted greater pressure than the FCT. Morales et al. (2021) support this perspective, suggesting that the NCT reflects higher insect pest pressure than the FCT. Based on these research findings, soybean genotypes identified as resistant in the NCT are likely to exhibit resistance in the field. Furthermore, a significant Pearson correlation (p = 0.034[*]; r = 0.345) was observed between the FCT and NCT. This finding underscores that these methods are consistent and provide complementary information, thereby strengthening the reliability and credibility of the study's results. The moderate strength of association indicated by the Pearson correlation coefficient (r = 0.345) highlights a meaningful relationship between the FCT and NCT methods.

Grouping of resistance in the FCT

Each soybean genotype exhibited varied resistance responses to *S. litura* across different plant ages post-infestation (Table 1). Genotypes consistently showing moderate to high resistance at all four ages were classified as resistant to *S. litura*. At 3 DAI, among the 38 tested soybean genotypes, the resistance response was predominantly categorized as moderately susceptible and susceptible, with 13 genotypes each, while only two were classified as highly resistant. By 6 DAI, the moderately resistant criterion became the most prevalent with 15 genotypes, and two genotypes remained categorized as highly resistant.

In the subsequent period (9 DAI), the number of genotypes classified as susceptible increased to 15 genotypes, while two genotypes were consistently categorized as highly resistant. At 12 DAI, the moderately resistant criterion was the most prevalent, encompassing 21 soybean genotypes. No genotypes exhibited highly resistant reaction, while five genotypes were classified as resistant, five showed susceptibility, and seven showed a high susceptibility. Considering the consistency of resistance reactions from the 38 soybean genotypes at 3, 6, 9, and 12 DAI based on the FCT, three soybean genotypes were identified, namely G17 (MR-MR-R-R), G14 (MR-MR-R-R), and G22 (R-HR-R-MR). Meanwhile, the resistance characteristics of the resistant control variety (G38), showed a resistance combination of HR-S-MR-R. Therefore, those soybean genotypes show promising potential for resistance against *S. litura*. A study by Adie et al. (2020) using FCT identified one resistant genotype, 11 genotypes with moderate resistance, and one genotype with moderate susceptibility when assessing for armyworm resistance. A genotype that is less favored for feeding can be considered resistant (Bayu et al., 2018). The preference can be linked to host plant attributes that influence insect-plant interactions, causing a substrate to act either as a stimulant, promoting feeding, or as a deterrent, preventing insect feeding and access (de Queiroz et al., 2020).

Table 1. Leaf damage intensity and its resistance category of 38 soybean advanced lines at four periods of observation under the free-choice test (FCT). GEN: Genotype, I: leaf damage intensity, HS: highly susceptible, S: susceptible, MR: moderately resistant, R: resistant, HR: highly resistant, SD: standard deviation.

| | Time of infestation (day after infestation, DAI) | | | | | | | |
| | 3 DAI | | 6 DAI | | 9 DAI | | 12 DAI | |
GEN	I (%)	Category	I (%)	Category	I (%)	Category	I (%)	Category
1	54.86	HS	35.75	MR	50.44	HS	48.94	S
2	37.87	S	44.54	HS	50.17	HS	37.10	MR
3	36.81	MR	38.20	S	41.61	S	47.13	S
4	58.75	HS	44.66	HS	41.95	S	41.86	MR
5	46.25	S	36.74	S	38.09	MR	41.63	MR
6	37.85	S	37.01	MR	32.42	MR	38.66	MR
7	35.54	MR	41.94	HS	39.97	S	42.53	MR
8	28.35	MR	42.19	HS	45.82	S	31.83	R
9	27.37	MR	35.90	MR	36.18	MR	33.70	R
10	41.90	S	32.48	MR	34.63	MR	42.94	MR
11	30.43	MR	38.31	S	41.12	S	42.63	MR
12	53.14	HS	36.89	MR	46.80	S	55.48	HS
13	50.31	S	39.57	S	47.80	S	53.98	HS
14	23.49	R	37.29	MR	24.32	HR	40.57	MR
15	33.74	MR	44.33	HS	45.25	S	42.16	MR
16	27.81	MR	37.61	S	38.83	MR	39.06	MR
17	29.16	MR	35.00	MR	28.14	R	34.46	R
18	52.84	HS	33.45	MR	37.84	MR	45.25	MR
19	37.89	S	42.37	HS	38.12	MR	38.03	MR
20	13.39	HR	36.71	MR	45.11	S	62.95	HS
21	28.57	MR	35.69	MR	35.57	MR	40.15	MR
22	21.07	R	27.08	HR	29.02	R	43.52	MR
23	54.17	HS	35.79	MR	34.46	MR	39.47	MR
24	38.75	S	35.70	MR	45.42	S	40.68	MR
25	38.25	S	29.55	R	35.88	MR	34.72	R
26	46.91	S	32.02	R	31.70	R	56.27	HS
27	42.28	S	40.73	S	45.10	S	51.25	S
28	49.40	S	40.68	S	50.18	HS	56.84	HS
29	47.44	S	38.23	S	40.91	S	42.22	MR
30	51.83	HS	34.95	MR	42.80	S	62.61	HS
31	62.90	HS	44.17	HS	48.67	HS	58.42	HS
32	35.57	MR	30.10	R	39.77	S	43.20	MR
33	28.99	MR	38.10	S	42.87	S	49.58	S
34	28.24	MR	36.93	MR	52.09	HS	43.29	MR
35	30.72	MR	28.24	HR	36.86	MR	39.95	MR
36	13.86	R	34.64	MR	18.94	HR	45.07	S
37	40.14	S	45.27	HS	33.87	MR	41.82	MR
38	0.00	HR	41.37	S	34.17	MR	28.33	R
Mean	37.28		37.37		39.55		44.16	
SD	13.28		4.53		7.41		8.10	

Grouping of resistance in the NCT

Evaluation of soybean genotype resistance responses to *S. litura* based on the NCT, imposes greater pressure on individual plants compared to the FCT (Table 2). After 3 DAI, among the 38 tested soybean genotypes, most of them were categorized as susceptible (16 genotypes), followed by moderately resistant (14 genotypes), highly susceptible (four genotypes), and two genotypes each classified as resistant and highly resistant. By 9 DAI, the distribution of resistance reactions for each soybean genotype changed to 15 genotypes classified as moderately resistant, 13 genotypes showed susceptibility, six showed high susceptibility, three were resistant, with only one genotype classified as highly resistant. At the end of the observation period, which was at 12 DAI,

the distribution of resistance criteria against *S. litura* showed 14 genotypes classified as susceptible, 11 genotypes showed moderate resistance, seven showed as high susceptibility, and six genotypes as resistant, with no soybean genotypes classified as highly resistant against *S. litura*.

Table 2. The leaf damage intensity and its resistance category of 38 soybean advanced lines at four periods of observation under the no-choice test (NCT). GEN: Genotype, I: leaf damage intensity, HS: highly susceptible, S: susceptible, MR: moderately resistant, R: resistant, HR: highly resistant, SD: standard deviation.

| | Time of infestation (d after infestation, DAI) | | | | | | | |
| | 3 DAI | | 6 DAI | | 9 DAI | | 12 DAI | |
GEN	I (%)	Category	I (%)	Category	I (%)	Category	I (%)	Category
1	45.28	S	46.57	MR	63.04	HS	57.60	S
2	43.45	MR	68.45	HS	55.06	S	59.03	HS
3	48.27	S	53.84	S	55.38	S	51.71	S
4	57.93	HS	59.52	HS	47.68	MR	59.31	HS
5	42.54	MR	46.58	MR	45.97	MR	53.01	S
6	37.92	R	42.66	MR	45.26	MR	59.11	HS
7	44.62	S	66.04	HS	44.59	MR	57.84	S
8	45.55	S	50.58	S	47.09	MR	46.56	MR
9	45.75	S	57.59	S	30.84	HR	46.03	MR
10	40.28	MR	56.70	S	48.78	MR	47.96	MR
11	42.81	MR	42.41	MR	48.87	MR	53.59	S
12	49.01	S	52.81	S	46.57	MR	50.95	MR
13	49.33	S	63.04	HS	46.33	MR	60.39	HS
14	56.42	HS	56.40	S	55.80	S	54.95	S
15	41.82	MR	35.02	R	44.24	MR	48.31	MR
16	49.58	HS	39.44	R	58.13	S	55.43	S
17	33.71	HR	40.06	R	38.58	R	39.45	R
18	44.44	MR	39.05	R	53.54	S	39.60	R
19	39.53	MR	34.85	R	43.06	R	38.99	R
20	46.23	S	39.88	R	53.32	S	54.88	S
21	46.83	S	50.48	S	35.91	R	34.08	R
22	48.21	S	50.42	S	63.54	HS	48.22	MR
23	50.32	HS	50.72	S	59.68	HS	59.03	HS
24	36.47	R	48.04	MR	61.16	HS	54.73	S
25	45.83	S	46.55	MR	46.68	MR	53.83	S
26	43.10	MR	43.47	MR	69.22	HS	66.28	HS
27	43.90	MR	45.72	MR	51.67	S	46.60	MR
28	45.31	S	57.14	S	56.37	S	50.61	MR
29	39.50	MR	49.15	MR	50.17	MR	53.73	S
30	39.34	MR	60.72	HS	62.75	HS	53.91	S
31	40.52	MR	50.46	S	52.13	S	53.77	S
32	45.61	S	48.96	MR	52.39	S	47.47	MR
33	46.95	S	50.35	S	48.49	MR	51.35	S
34	48.73	S	46.87	MR	49.55	MR	49.07	MR
35	42.43	MR	39.58	R	46.90	MR	37.41	R
36	45.35	S	48.26	MR	56.89	S	59.08	HS
37	44.24	MR	50.92	S	52.13	S	54.94	MR
38	32.92	HR	66.25	HS	51.27	S	41.02	R
Mean	44.47		49.88		51.03		51.31	
SD	5.09		8.39		7.71		7.15	

Considering these findings, the resistance profile of the 38 soybean genotypes is predominantly characterized by moderately resistant to susceptible categories. The soybean genotypes tested for their resistance reactions to *S. litura* were the result of selection from the recombination of armyworm-resistant parents with high-yielding soybeans. Considering the resistance responses to *S. litura* over four age periods (3,

6, 9, and 12 DAI), two soybean genotypes were identified: G17 with a resistance combination of HR-R-R-R and G19 categorized as MR-R-R-R. The genotype G38, used as a resistant control against *S. litura*, exhibited the resistance combination HR-S-MR-R. Therefore, G17 and G19 can be considered to have a resistant response to *S. litura* based on the NCT. The soybean genotypes G17 exhibited consistent resistance in both NCT and FCT. A study by Mathpal et al. (2022) also found a soybean germplasm (RSC 11-03) as resistant to *S. litura* based on the FCT and NCT, indicated by its minimal leaf area consumption. Meanwhile, a similar study by Adie et al. (2020) found that two genotypes (19 BE and G100H) exhibited moderate resistance against *S. litura*.

In this study, each soybean genotype exhibited varying resistance levels in both the NCT and FCT. Similarly, previous studies have demonstrated varying resistance responses to armyworm among different soybean cultivars based on the choice and no-choice tests (Krisnawati et al., 2017; Bayu et al., 2018; Adie et al., 2020; Mathpal et al., 2022). The variation in the degree of plant resistance to insect pests is closely linked to the presence of various defensive compounds and physical traits in plants (Wani et al., 2022). Research has shown that higher levels of specific secondary metabolites, such as phenolic compounds and flavonoids, play a crucial role in deterring armyworms (Singh and Kariyat, 2020; Chatterjee et al., 2023). Furthermore, physical characteristics such as trichomes and tougher leaf tissues contribute to making soybean plants less palatable and more difficult for pests to digest, thereby reducing the damage inflicted (Abebe, 2021).

The performance of agronomic traits

The ANOVA for seed yield and agronomic characteristics (Table 3) in the FCT showed that all agronomic traits displayed significant differences among the tested soybean genotypes, while the number of empty pods (NEP) did not show significant differences. Conversely, in the NCT, all these agronomic traits exhibited significant differences among the tested soybean genotypes.

Table 3. Analysis of variance of 38 soybean genotypes for yield and yield components. ns: nonsignificant; *significant at 5% probability level ($p < 0.05$); **significant at 1% probability level ($p < 0.01$). FCT: Free-choice test; NCT: no-choice test.

		Mean square			
		FCT		NCT	
Nr	Character	Replicate	Genotype	Replicate	Genotype
1	Plant height, cm	194.3048**	142.6609**	10.1293ns	135.1018**
2	Number of branches per plant	1.3771ns	1.8990**	1.2368ns	3.0967**
3	Number of nodes per plant	1.2719ns	16.3598**	0.9298ns	38.1137**
4	Number of filled pods per plant	55.0614ns	68.0907**	7.3333ns	182.1237**
5	Number of empty pods per plant	2.7609ns	2.3472ns	1.5855ns	5.5656**
6	100 seed weight, g	41.6905*	7.9594**	0.3157ns	16.8433**
7	Seed yield, g plant^{-1}	6.2534**	78.4633**	0.1698ns	10.1827**

The yield and yield components of 38 soybean genotypes in the FCT and NCT are presented in the Figure 2. Performance of supporting production characteristics (plant height, number of branches and number of nodes) differs between test environments (FCT and NCT). Plant height in the FCT is higher compared to the NCT, whereas number of branches and number of nodes are higher in the NCT. Regarding productivity traits of number of filled pod, number of empty pods, and 100 seed weight, it appears that all are higher in the NCT compared to the FCT. The cumulative effect of these agronomic characteristics results in an average seed yield per plant in the NCT (8.07 g) being significantly higher than in the FCT (5.85 g).
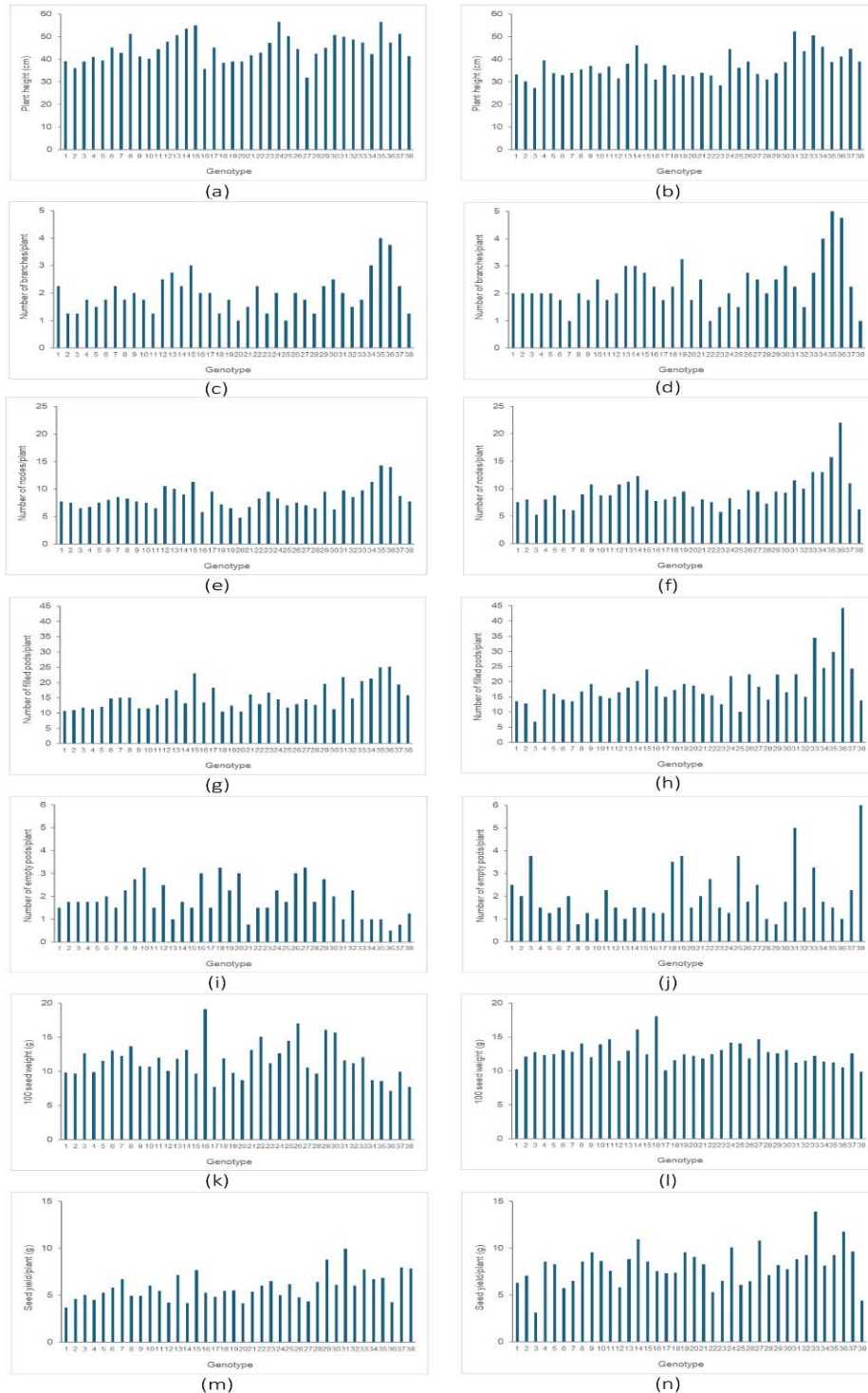
Figure 2. Performance of the agronomic traits of 38 soybean genotypes: Plant height in the free-choice test (FCT) (a), plant height in the no-choice test (NCT) (b), number of branches per plant in the FCT (c), number of branches per plant in the NCT (d), number of nodes per plant in the FCT (e), number of nodes per plant in the NCT (f), number of filled pods per plant in the FCT (g), number of filled pods per plant in the NCT (h), number of empty pods per plant in the FCT (i), number of empty pods per plant in the NCT (j), 100 seed weight in the FCT (k), 100 seed weight in the NCT (l), seed yield per plant in the FCT (m), seed yield per plant in the NCT (n).

The association between seed yield and yield components in the FCT environment is presented in Figure 3. In the FCT, soybean seed yield per plant (SYP) is strongly influenced by plant height (PHG) ($r = 0.357^*$), filled pods per plant (NFP) ($r = 0.542^{**}$), and number of empty pods per plant (NEP) ($r = - 0.360^*$) (Figure 3). This suggests that increasing plant height and filled pods per plant enhances seed yield, whereas increasing the empty pods per plant decreases seed productivity in soybeans. Supporting traits that contribute to a higher number of filled pods per plant include plant height, branches number (NOB), and nodes (NON). It is also evident that plant height, number of branches, nodes, and filled pods mutually support each other.

Figure 3. Correlogram (correlation matrix) for yield and yield components of 38 soybean genotypes in the free-choice test (FCT) environment. Blue color indicates positive correlations, while red color indicates negative correlations. The intensity of the color reflects the strength of the correlation: darker colors indicate stronger correlations. SYP: seed yield per plant; PHG: plant height; NOB: number of branches; NON: number of nodes; NFP: number of filled pods; NEP: number of empty pods; HSW: 100-seed weight.

The relationship patterns between seed yield and agronomic traits in the NCT environment reveals a similar pattern to that observed in the FCT environment (Figure 4). The seed yield exhibited positive significant correlation with plant height ($r = 0.601^{**}$), branches per plant ($r = 0.510^{**}$), nodes per plant ($r = 0.658^{**}$), and filled pods per plant ($r = 0.731^{**}$). Meanwhile, the number of empty pods has less influence on seed yield. The interdependence among four traits (plant height, branches per plant, nodes per plant, and filled pods per plant) is also evident in the NCT environment. Recent studies have highlighted a significant positive correlation between seed yield and several key plant morphological traits in soybean (Li et al., 2020; Lodhi et al., 2023). This result suggests their potential utility as direct selection criteria for enhancing yield.

Figure 4. Correlogram (correlation matrix) for yield and yield components of 38 soybean genotypes in the no-choice test (NCT) environment. Blue color indicates positive correlations, while red color indicates negative correlations. The intensity of the color reflects the strength of the correlation: Darker colors indicate stronger correlations. SYP: seed yield per plant; PHG: plant height; NOB: number of branches; NON: number of nodes; NFP: number of filled pods; NEP: number of empty pods; HSW: 100-seed weight.

Selection of agronomic traits and armyworm resistance

A soybean genotype distinguished by agronomic traits that improve both high seed yield and tolerance to specific insect pests represents an ideal genotype. In this study, the "which won where" Genotype by Trait biplot is used to determine the best-performing genotypes on the basis of multiple traits (Yan and Rajcan, 2002). The selection of the best-performing genotypes is based on the data in the NCT environment due to its higher resistance pressure compared to FCT. The "which won where" GT biplot (Figure 5) visualizes the relationships among different soybean genotypes and traits based on their principal components (PC1 and PC2), which explain 51.59% and 18.05% of the variance, respectively. The biplot is divided into sectors/quadrants, with each quadrant containing the traits and genotypes that perform best in those conditions. The vertices of the polygon formed by the outermost genotypes indicate the best-performing genotypes for the traits in that quadrant.

According to Figure 5, the biplot is divided into six quadrants, which the distribution of agronomic traits is observed in only two quadrants (quadrant II and quadrant V). There were no specific traits located at quadrants I, III, IV, and V, indicating genotypes in here have average or less significant performance across the traits. In quadrant II, genotypes located in this quadrant exhibit higher values for the traits of number of branches, number of nodes, number of filled pods, plant height, and 100-seed weight (HSW), all of which are positively correlated. Genotype 36, located at the vertices of the polygon in quadrant II, stands out as the top performer for number of branches, number of nodes, number of filled pods, plant height, and 100-seed weight, despite being relatively less resistant to *S. litura*. In quadrant IV, genotypes exhibit potential superiority in yield but also display a high intensity of leaf damage (RES), indicating susceptibility to *S. litura*. Genotype 16 emerges as the top performer for high yield but susceptible to *S. litura*. In the which-won-where of GT biplot, genotypes positioned in diametrically opposite quadrants (such as quadrant IV and quadrant VI) are at the furthest extremes from each other with respect to the traits measured. These positions imply contrasting performance in the traits located within those quadrants. In Figure 5, genotypes in quadrant VI demonstrate a high resistance to *S. litura*, but comparatively lower yield performance. Genotypes 38 (resistant control variety) and genotype 17 exhibited the best performance for resistance to *S. litura*.

The application of GT biplot analysis has proven to be effective in identifying superior soybean genotypes based on multiple traits. Recent studies demonstrated the utility of GT biplot in evaluating multiple traits simultaneously, such as abiotic resistance and agronomic traits (Adie et al., 2022; Ruswandi et al., 2023). In this

study, based on the which-won-where biplot and the leaf damage intensity in both NCT and FCT, genotypes 17 and 19 exhibited notable resistance to *S. litura*, with their positioning as promising candidate for breeding programs focused on enhancing this trait. The development of resistant varieties against armyworm has proven to be a sustainable and eco-friendly approach in pest management systems, demonstrating effectiveness in pest control and minimizing insecticide use.
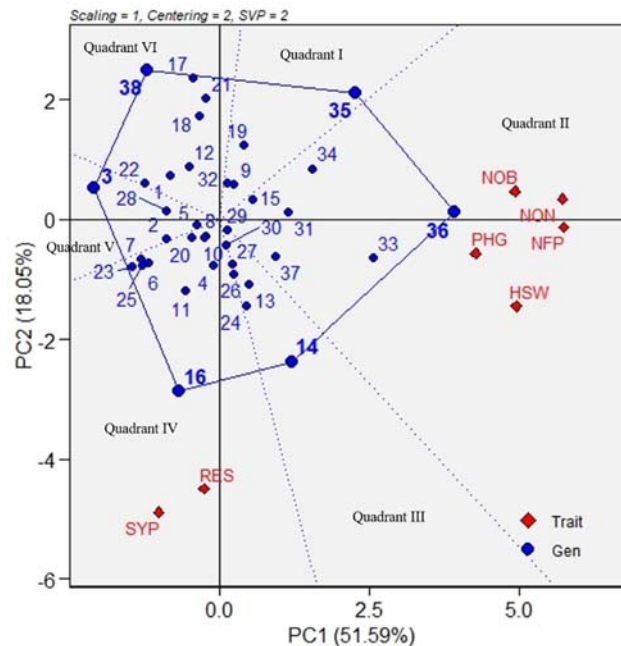

Figure 5. The polygonal representation of the biplot depicting genotype-trait interactions identifies which cultivars exhibited superior values in specific traits. SYP: seed yield per plant; PHG: plant height; NOB: number of branches; NON: number of nodes; NFP: number of filled pods; HSW: 100-seed weight; RES: intensity of leaf damage; PC1: principal component 1; PC2: principal component 2; Gen: Genotype.

CONCLUSIONS

The resistance responses of soybean advanced lines to armyworm *Spodoptera litura* vary among cultivars and across test environments (FCT and NCT). The no-choice test (NCT) imposes a higher intensity of leaf damage compared to the free-choice test (FCT). Two genotypes (17 and 19) consistently showed resistance to *S. litura* across various observation periods. Seed yield in soybeans is determined by the plant height, number of filled pods, number of nodes, and number of branches. The selection based on multiple traits of Genotype by Trait (GT) Biplot revealed that genotype 36 is the best performer for plant height, number of filled pods, number of nodes, and number of branches, and 100 seed weight, despite being relatively less resistant to *S. litura*. Furthermore, genotypes 38 (resistant-control variety) and genotype 17 showed the best performance for resistance to *S. litura*, although they did not exhibit significantly high seed yields, unlike genotype 16, which has a higher yield but is susceptible to armyworm. Nonetheless, genotypes 17 and 19 show potential as valuable sources of soybean resistance genes against armyworm, making them promising candidates for breeding programs aimed at improving this trait.

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